Construction of Xylose Utilizing *Zymomonas mobilis* Integrants Based on Strain ATCC31821

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Abstract

Strain Development Team at NREL has successfully integrated the four genes (*xylA*, *xylB*, *tal* and *tkt*) necessary for xylose utilization into the genome of *Zymomonas mobilis* ATCC 31821- a more desirable ethanologen by industries for its properties such as tolerance to higher temperature, higher sugar feed streams and acetic acid. Two sets of genes were sequentially integrated in the genome of strain 31821 using homologous recombination and transposition methods. The fermentation performance of the integrants was similar to the plasmid-bearing parental strains.

Introduction

Z. mobilis has been recognized as a viable alternative to yeast for ethanol production from biomass for its high conversion yield, high ethanol tolerance, resistance to pretreated hardwood hydrolysate and fermentation capability at lower pH. In the previous research of *Zymomonas* metabolic engineering, we developed genomically integrated *Z. mobilis* ATCC39676 based strains, which can ferment pentose sugars (D-xylose and L-arabinose) and glucose simultaneously to ethanol.

While ATCC39676 based strains are suitable for the SSCF process (e.g. corn stover hydrolysate) in which total sugar concentration is moderate and glucose very low, we found that a plasmid-bearing, xylose-fermenting ATCC31821 strain demonstrated superior performance in hydrolysates containing high soluble sugars (such as corn fiber and rice straw) and was tolerant to higher temperature and acetic acid. We now report the process of gene integration of xylose utilization pathway in the genome of *Z. mobilis* ATCC31821 and preliminary fermentation evaluation for the integrants.

What Genes to Integrate in the Genome of 31821?

Operons- P_{eno}*taltkt* or P_{gap}*taltkt*

1. transketolase gene (*tkt*)
2. transaldolase gene (*tal*)

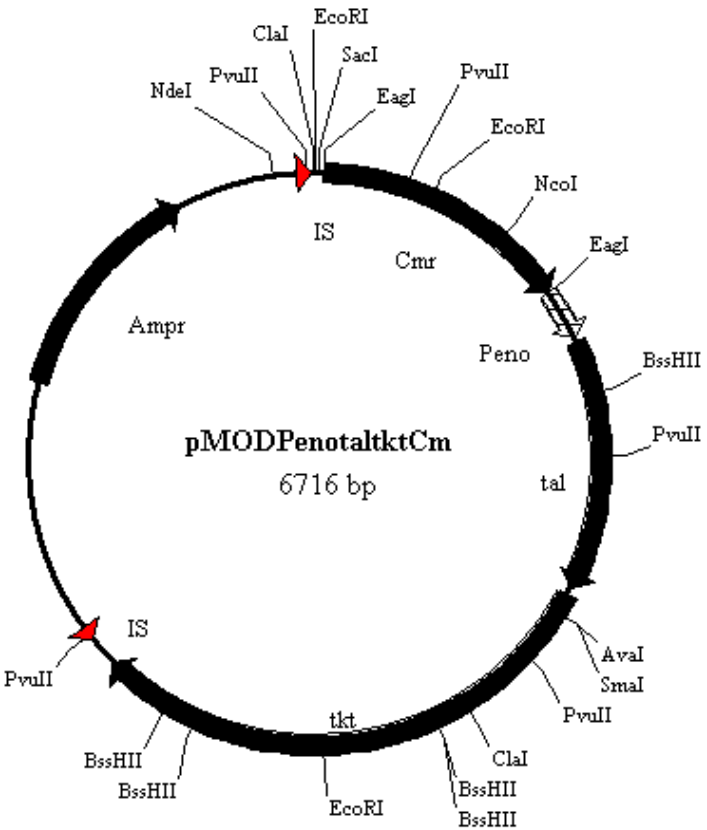
Operon-P_{gap}*xylAB*

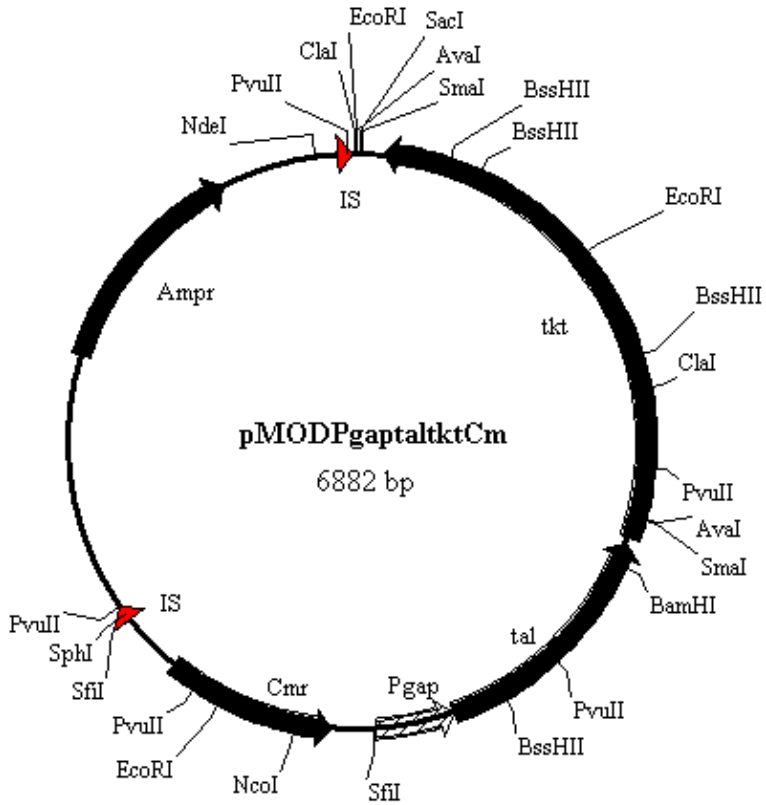
3. xylose isomerase gene (*xylA*)
4. xylulokinase gene (*xylB*)

Integration in *Z. mobilis* 31821

- Homologous Recombination
  - –Lactate dehydrogenase gene (*ldh*)
- Transposition
  - –Random insertion in the genome

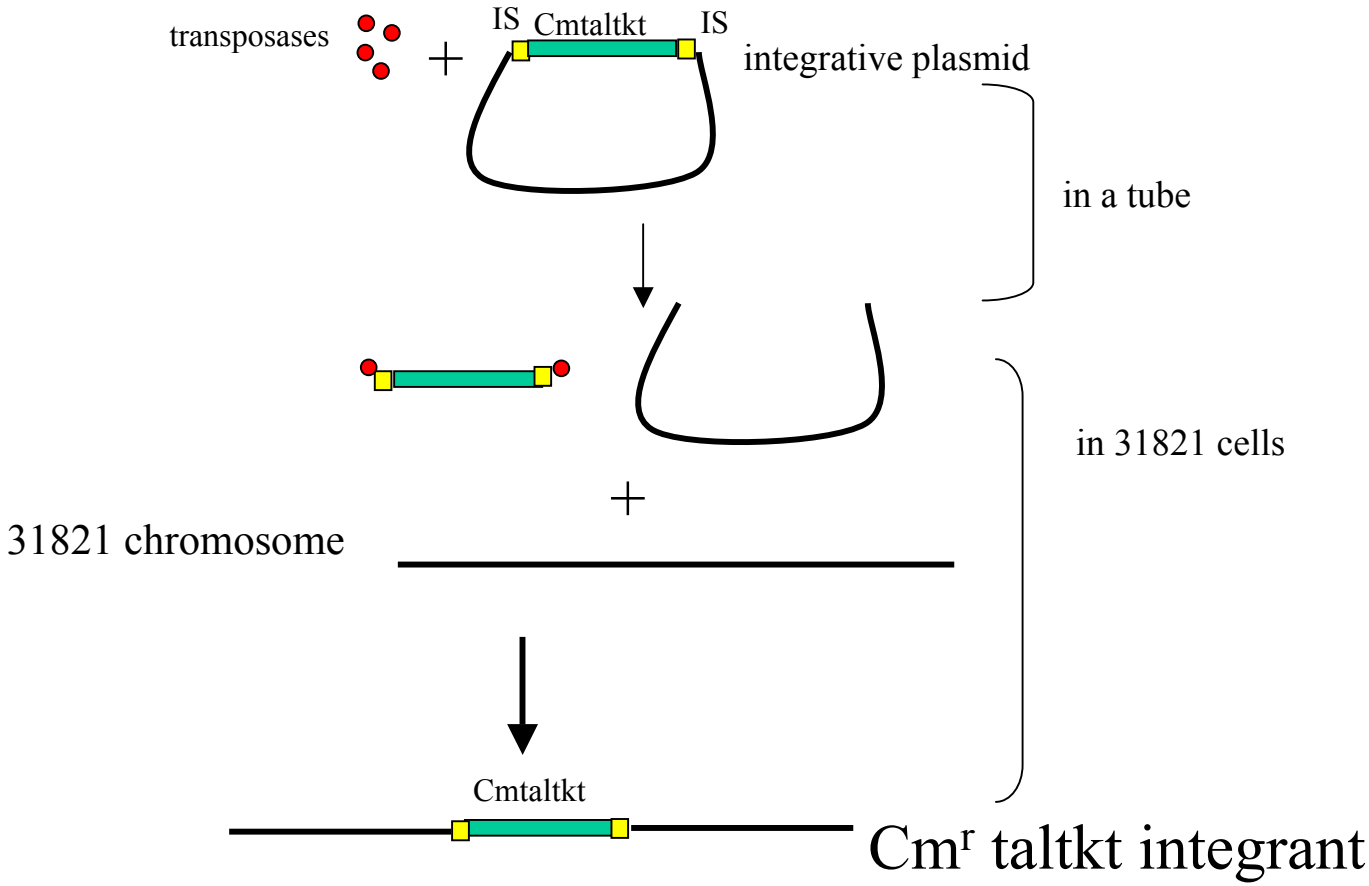
Integrative Plasmids for Transposition of *taltkt*





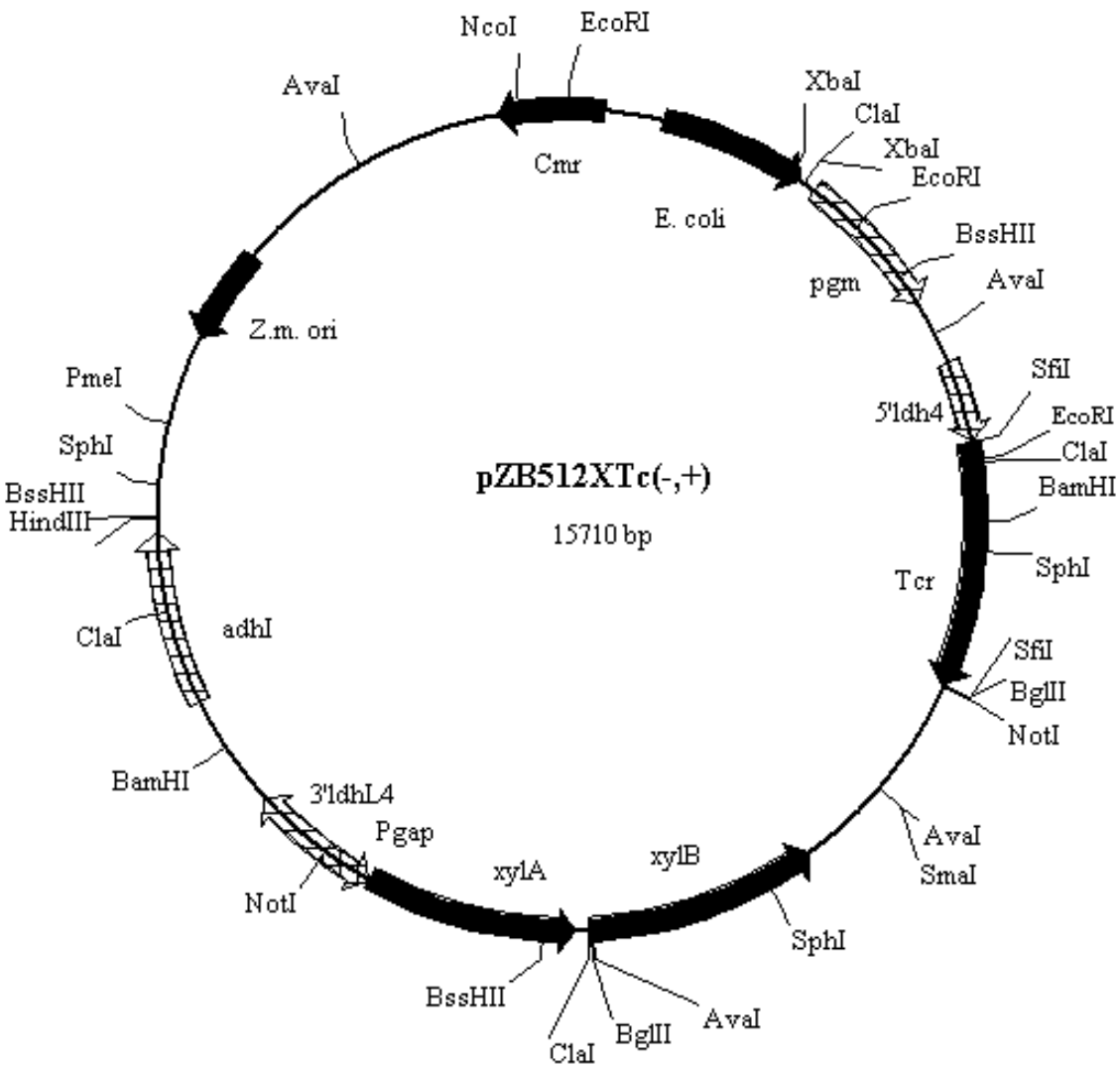
Integration by *In Vitro* Transposition

Host: *Z. m*. 31821
Integrative plasmids: pMODPenotaltktCm or pMODPgaptaltktCm



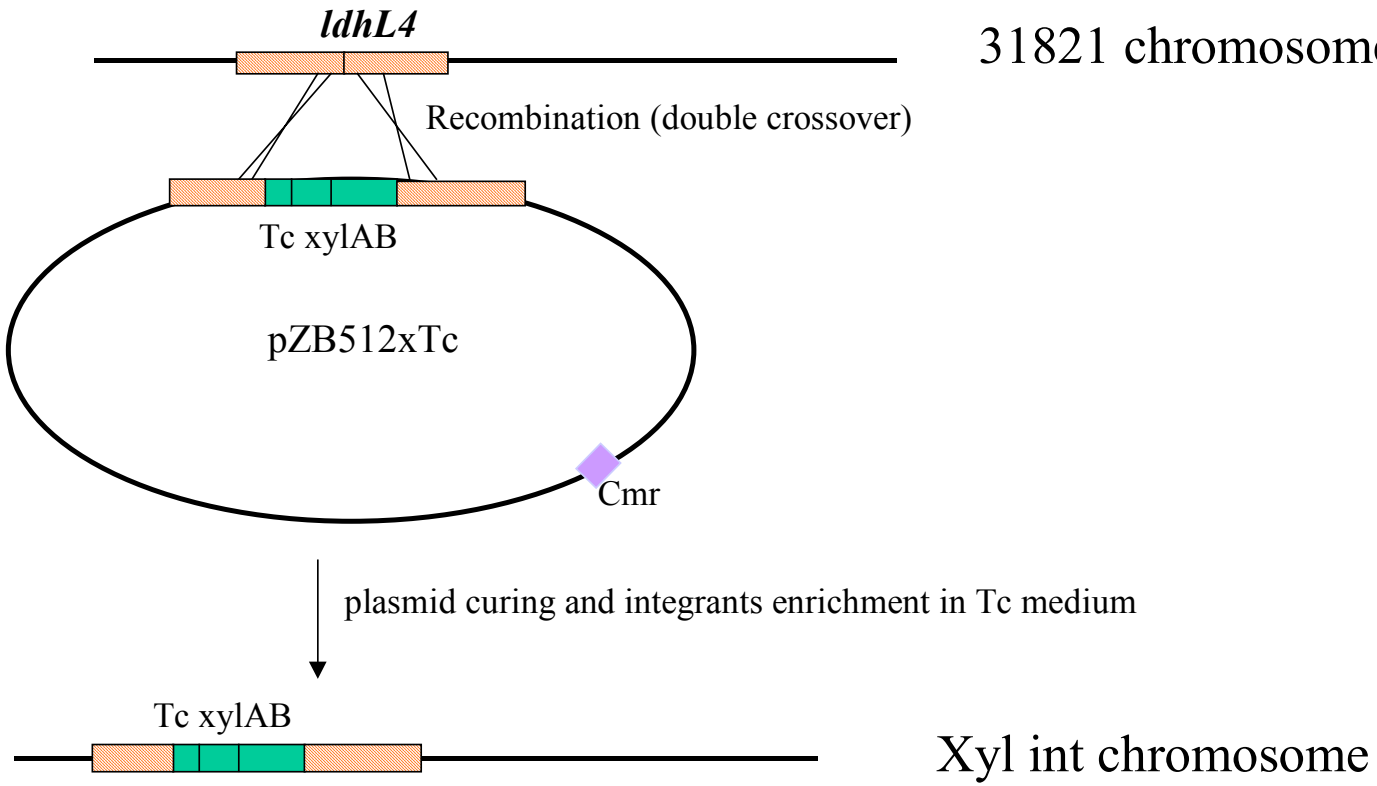
•Transposases and pMOD vector (EZ::TN™) were purchased from Epicentre.

Integrative Plasmid for Homologous Recombination of P_{gap}*xylAB*



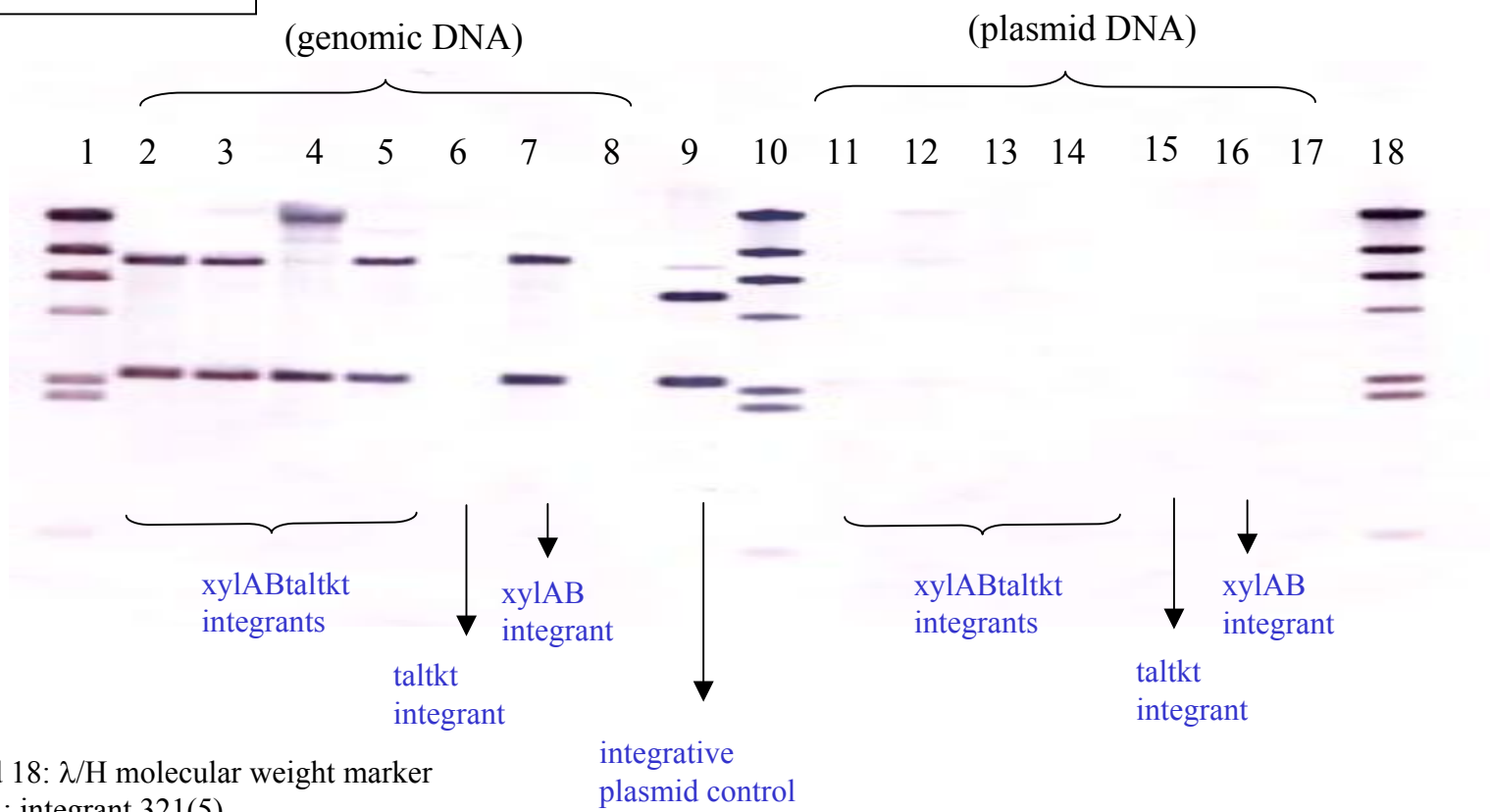
Integration by Homologous Recombination

Host: *Z. m*. 31821
Integrative plasmid: pZB512xTc



Southern Blot for the Integrants DNA

A. **-DIG-xylB probe**
-SphI digestion



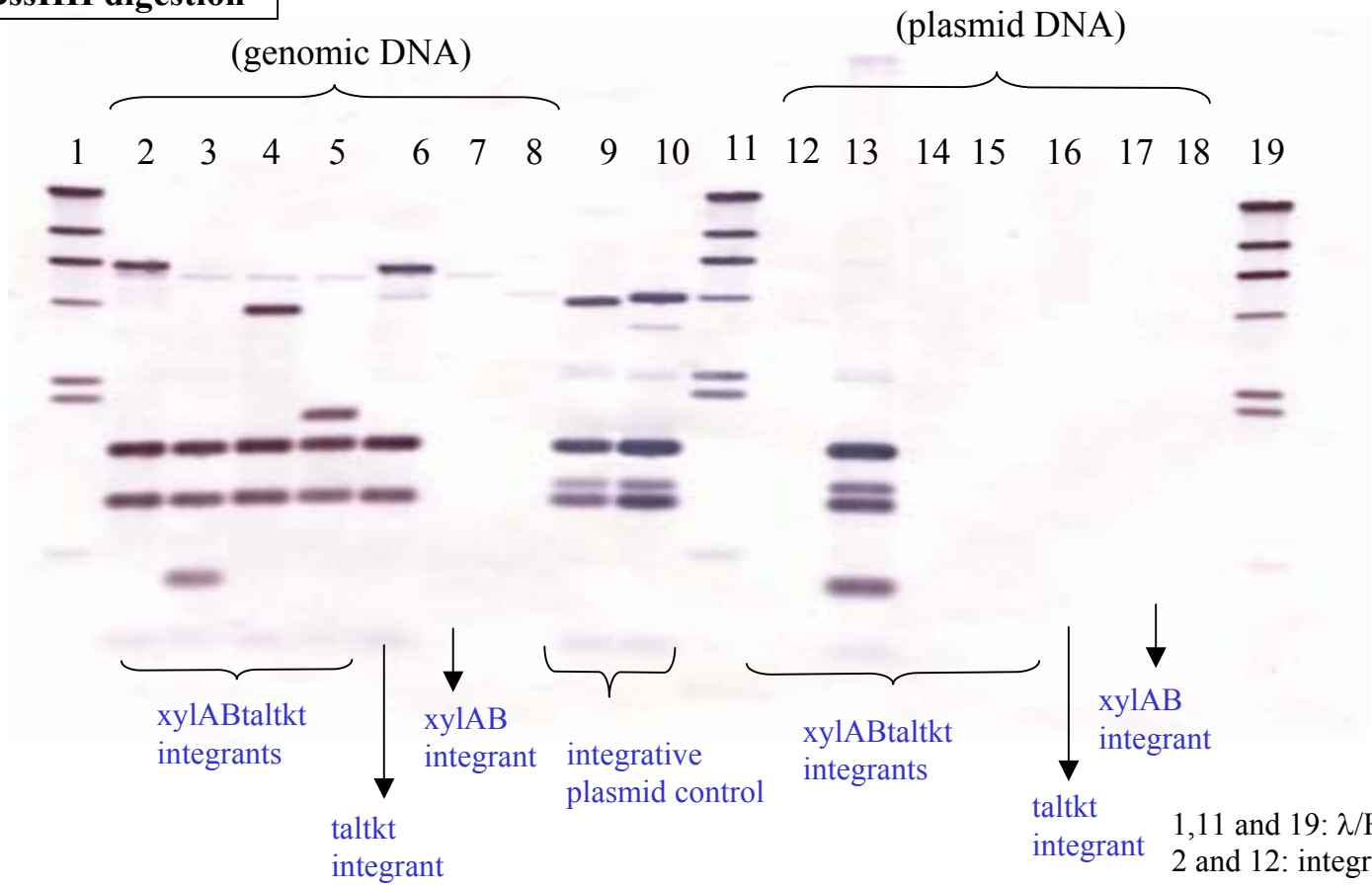
1,10 and 18: λ/H molecular weight marker
2 and 11: integrant 321(5)
3 and 12: integrant 481
4 and 13: integrant 2032
5 and 14: integrant 2122
6 and 15: integrant int2
7 amd 16: integrant x9i
8 and 17: ATCC31821 host

Southern Blot for the Integrants DNA

B.

-DIG-tkt probe

-BssHII digestion



1,11 and 19: λ/H molecular weight marker
2 and 12: integrant 321(5)
3 and 13: integrant 481
4 and 14: integrant 2032
5 and 15: integrant 2122
6 and 16: integrant int2
7 amd 17: integrant x9i
8 and 18: ATCC31821 host

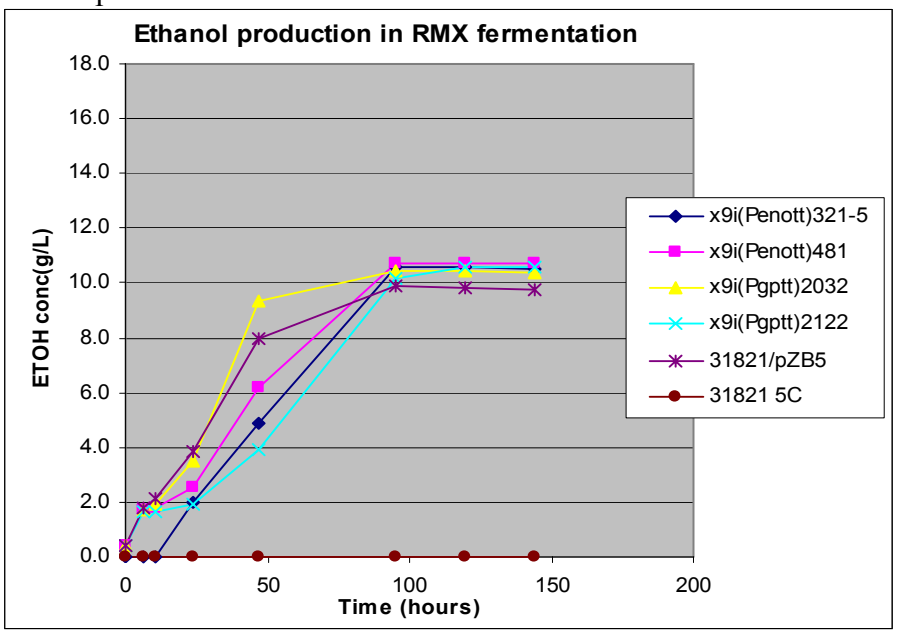
Enzymatic Activity for the Integrants

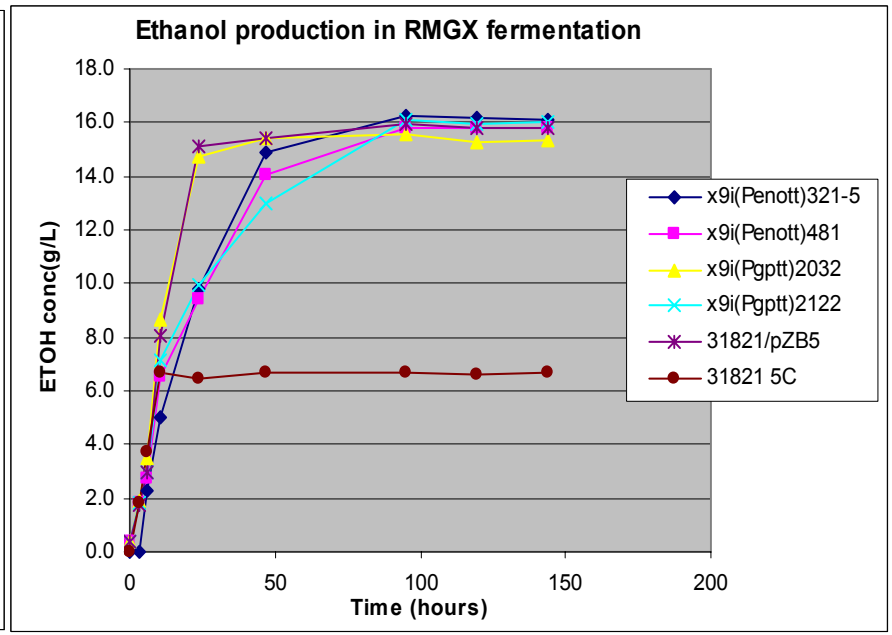
	Specific Activity (umol/min-mg protein)			
	XI	**XK**	**TAL**	**TKT**
321(5)	0.06	0.17	2.10	0.91
481	0.04	0.23	2.27	0.74
2032	0.15	0.23	1.76	1.05
2122	0.04	0.19	1.56	0.51
int2	0.02	0.02	1.19	0.31
x9i	0.04	0.06	ND*	0.03
31821/pZB5	0.06	0.55	2.52	1.78

321(5), 481, 2032 and 2122: xylABtaltkt integrants
int2 : taltkt integrant
x9i : xylAB integrant
31821/pZB5: xylABtaltkt plasmid-bearing strain
ND: not detected

Fermentation Evaluation of the Integrants

Medium: RM supplimented with xylose (2%) or xylose(2%) and glucose (1%) mixture
Volume: 80 mL in 100-mL bottle, static
pH: not controlled
Temp: 30°C





Conclusion

- **The four genes necessary for xylose utilization were successfully integrated in the *Z. mobilis* ATCC31821 genome using both homologous recombination and transposition.**

- **The genes were expressed and the fully integrated *Z. mobilis* strains were capable of growing on xylose as a sole carbon source.**

- **Preliminary analysis indicated that integrants were able to ferment xylose as well as glucose to ethanol at similar yields.**

Acknowledgement

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